

LAP17 Rec'd PCT/PTO 28 APR 2006

In the ClaimsWe claim:

Claims 1-33 (cancelled)

Claim 34 (New): A composition of matter comprising:

(a) a liposome comprising a first adjuvant and at least one second adjuvant, which is different from the first adjuvant, and at least one therapeutic agent; or

(b) a mixture of liposomes, said mixture comprising at least a first adjuvant and at least one therapeutic agent and at least a second liposome comprising at least a second adjuvant, which is different from the first adjuvant; or

(c) a mixture of liposomes comprising a first liposome comprising at least a first adjuvant, a second liposome comprising at least one therapeutic agent and at least a third liposome comprising at least a second adjuvant, which is different from the first adjuvant; or

(d) mixture of liposomes comprising a first liposome comprising at least a first adjuvant, a second liposome comprising at least one therapeutic agent and a liquid medium comprising at least a second adjuvant, which is different from the first adjuvant; or

(e) a liposomal composition comprising a liposome comprising a first adjuvant and at least one therapeutic agent and a liquid medium comprising at least a second adjuvant, which is different from the first adjuvant.

Claim 35 (New): The composition of matter of claim 34, wherein the liposomes of (a) – (c) are in a liquid medium.

Claim 36 (New): The composition of matter of claim 35, wherein the liquid medium of (a) – (e) is selected from the group consisting of H₂O, aqueous salt solution, and buffer solution.

Claim 37 (New): The composition of matter of claim 34, wherein (a) – (e) further comprise at least one further component selected from the group consisting of an adjuvant, an additive, and an auxiliary substance.

Claim 38 (New): The composition of matter of claim 34, wherein the lipids of the liposomes comprise cholesterol and at least one negatively charged lipid.

Claim 39 (New): The composition of matter of claim 38, wherein the negatively charged lipid comprised in the liposome is selected from the group consisting of phosphatidylserine (PS), phosphatidylglycerol (PG), and phosphatidic acid (PA).

Claim 40 (New): The composition of matter of claim 34, wherein the liposomes of (a) – (e) comprise cholesterol and at least two components selected from the group consisting of PS, PG, and PE.

Claim 41 (New): The composition of matter of claim 40, wherein in relation to the total molar lipid composition of the liposome, each liposome comprises:

- a) between 20 mol% and 60 mol% CH; and
- b) between 20 mol% and 50 mol% PS;
between 20 mol% and 50 mol% PG and
between 20 mol% and 50 mol% PE, respectively.

Claim 42 (New): The composition of matter of claim 38, wherein between one and three components selected from the group consisting of CH, PS, PG and PE are present in relation to the total molar lipid composition of the liposome at a molar ratio of between 30 mol% and 36 mol%.

Claim 43 (New): The composition of matter of claim 38, wherein the remaining lipid of the liposome is selected from the group consisting of glycerides, glycerophospholipides, glycerophosphinolipids, glycerophosphonolipids, sulfolipids, sphingolipids, phospholipids, isoprenolides, steroids, stearines, sterols and carbohydrate containing lipids.

Claim 44 (New): The composition of matter of claim 43, wherein said remaining phospholipid is phosphatidylcholine (PC) or PE.

Claim 45 (New): The composition of matter of claim 40, wherein the lipids of the liposome consist essentially of CH, PS, and PG; CH, PS, and PE; CH, PG, and PE; or CH, PG, PS, and PE.

Claim 46 (New): The composition of matter of claim 34, wherein the therapeutic agent is selected from the group consisting of a drug and an antigen.

Claim 47 (New): The composition of matter of claim 46, wherein the antigen is selected from the group of antigens consisting of a tumor antigen, a viral antigen, a fungal antigen, a bacterial antigen, an autoimmune antigen, and an allergen.

Claim 48 (New): The composition of matter of claim 47, wherein the tumor antigen is selected from the group consisting of T-cell-defined cancer-associated antigens belonging to unique gene products of mutated or recombined cellular genes, Cancer-testis (CT) antigens, Tumor virus antigens, overexpressed or tissue-specific differentiation antigens, and widely expressed antigens; or fragments or derivatives of any of the foregoing.

Claim 49 (New): The composition of matter of claim 48, wherein the tumor antigen is selected from the group consisting of cyclin-dependent kinase 4 (CDK4), p15^{Ink4b}, p53, AFP, β -catenin, caspase 8, p53, p21^{Ras} mutations, Bcr-abl fusion product, MUM-1 MUM-2, MUM-3, ELF2M, HSP70-2M, HST-2, KIAA0205, RAGE, myosin/m, 707-AP, CDC27/m, ETV6/AML, TEL/Aml1, Dekcain, LDLR/FUT, Pml-RAR α , TEL/AML1, NY-ESO-1, members of the MAGE-family (MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-10, MAGE-12), BAGE, DAM-6, DAM-10, members of the GAGE-family (GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7B, GAGE-8), NA-88A, CAG-3, RCC-associated antigen G250, human papilloma virus (HPV)-derived E6 E7 oncoproteins, Epstein Barr virus EBNA2-6, LMP-1, LMP-2, gp77, gp100, MART-1/Melan-A, p53, tyrosinase, tyrosinase-related protein (TRP-1 and TPR-2), PSA, PSM, MC1R, ART4, CAMEL, CEA, CypB, HER2/neu, hTERT, hTRT, iCE, Muc1, Muc2, PRAME RU1, RU2, SART-1, SART-2, SART-3, and WT1.

Claim 50 (New): The composition of matter of claim 46, wherein the antigen is derived from a virus selected from the group of virus consisting of Retroviridae, Picornaviridae,

enterovirus, Calciviridae, Togaviridae, Flaviridae, Coronaviridae, Rhabdoviridae, Filoviridae, Paramyxoviridae, Orthomyxoviridae, Bungaviridae, Arena viridae, Reoviridae, Birnaviridae, Hepadnaviridae, Parvovirida, Papovaviridae, Adenoviridae, Herpesviridae, Poxviridae, Iridoviridae, and Hepatitis C virus.

Claim 51 (New): The composition of matter of claim 50, wherein the antigen is derived from a virus selected from the group consisting of HIV-1, HIV-LP, polio virus, hepatitis A virus, human coxsackie virus, rhinovirus, echovirus, a strain of Calciviridae that causes gastroenteritis, equine encephalitis virus, rubella virus, dengue virus, encephalitis virus, yellow fever virus, coronavirus, vesicular stomatitis virus, rabies virus, Ebola virus, Marburg virus, parainfluenza virus, mumps virus, measles virus, respiratory syncytical virus, influenza virus, Hantaan virus, bunga virus, phlebovirus, Nairo virus, hemorrhagic fever virus, reovirus, orbivirus, rotavirus, parvovirus, papilloma virus, simian virus-40 (SV40), polyoma virus, herpes simplex virus (HSV) 1, HSV 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus, variola virus, vaccinia virus, pox virus, Hepatitis B virus, and African swine fever virus.

Claim 52 (New): The composition of matter of claim 47, wherein the fungal antigen is derived from a fungus selected from the group consisting of *Cryptococcus* species, *Histoplasma* species, *Coccidioides* species, *Blastomyces* species, *Chlamydia* species, and *Candida* species,

Claim 53 (New): The composition of matter of claim 52, wherein the fungal antigen is derived from a fungus selected from the group consisting of in particular *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Chlamydia trachomatis*, and *Candida albicans*.

Claim 54 (New): The composition of matter of claim 47, wherein the bacterial antigen is derived from a bacterium selected from the group consisting of Helicobacter species, Borelia species, Legionella species, Mycobacteria species, Staphylococcus species, Niesseria species, Listeria species, Streptococcus species, anaerobic Streptococcus species, pathogenic Campylobacter species, Enterococcus species, Haemophilus species, Bacillus species, Corynebacterium species, Erysipelothrix species, Clostridium species, Enterobacter species, Klebsiella species, Pasturella species, Bacteroides species, Fusobacterium species,

Streptobacillus species, Treponema species, Leptospira, pathogenic Escherichia species, and Actinomyces species.

Claim 55 (New): The composition of matter of claim 54, wherein the bacterial antigen is derived from a bacterium selected from the group consisting of *Helicobacter pylori*, *Borrelia burgdorferi*, *Legionella pneumophila*, *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. goodii*, *Staphylococcus aureus*, *N. gonorrhoeae*, *N. meningitidis*, *Listeria monocytogenes*, *S. pyogenes*, *S. agalactiae*, *S. faecalis*, *S. bovis*, *S. pneumoniae*, *Haemophilus influenzae*, *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Erysipelothrix rhusiopathiae*, *C. perfringens*, *C. tetani*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pasteurella multocida*, *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pertense*, and *Actinomyces israelii*.

Claim 56 (New): The composition of matter of claim 34, wherein the first and second adjuvant is selected from the group consisting of unmethylated DNA, bacterial products from the outer membrane of Gram-negative bacteria, synthetic lipopeptide derivatives, heat shock proteins (HSP), lipoarabinomannan, peptidoglycan, zymosan, dsRNA or synthetic derivatives thereof, polycationic peptides, taxol, fibronectin, flagellin, imidazoquinoline, cytokines with adjuvant activity, 25-dihydroxyvitamin D3 (calcitriol), synthetic oligopeptides, and gel-like precipitates of aluminum hydroxide (alum).

Claim 57 (New): The composition of matter of claim 56, wherein the first and second adjuvant is selected from the group consisting of CpG ODN with phosphorothioate (PTO) backbone (CpG PTO ODN), CpG ODN with phosphodiester (PO) backbone (CpG PO ODN), monophosphoryl lipid A (MPLA), lipopolysaccharides (LPS), muramyl dipeptides or derivatives thereof, Pam₃Cys, Poly I:poly C, HSP 70, poly-L-arginine, GM-CSF, interleukin- (IL-)2, IL-6, IL-7, IL-18 type I, IL-18 type II, interferon-gamma, TNF-alpha, and MHCII-presented peptides.

Claim 58 (New): The composition of matter of claim 34, wherein the first and the second adjuvant stimulate different receptors and/or pathways within cells of the immune system.

Claim 59 (New): The composition of matter of claim 58, wherein the first and the second adjuvant stimulate at least two receptors selected from the group consisting of type I cytokine

receptors, type II cytokine receptors, TNF receptors, vitamin D receptor acting as transcription factor, Toll-like receptor 1 (TLR-1), TLR-2, TLR 3, TLR4, TLR5, TLR-6, TLR7, and TLR9.

Claim 60 (New): The composition of matter of claim 59, wherein the first and the second adjuvant, which primarily stimulate different receptors, are selected from among:

- a) type I cytokine receptors selected from the group consisting of GM-CSF, IL-2, IL-6, and IL-7;
- b) type II cytokine receptors selected from the group consisting of IFN- α/β and IFN- γ ;
- c) TNF receptors selected from the group consisting of TNF- α and CD40 ligand;
- d) vitamin D receptor calcitriol;
- e) TLR-1 selected from the group consisting of tri-acyl lipopeptides from the bacteria or mycobacteria, and soluble factors from *Neisseria meningitides*:
- f) TLR-2 selected from the group consisting of lipopeptides, lipoarabinomannan from mycobacteria, peptidoglycan, zymosan, heat shock proteins (HSPs), lipoteichoic acid from gram-positive bacteria, phenol-soluble modulin from *Staphylococcus* species, glycoinositolphospholipids from *Trypanosoma* species, glycolipids from *Treponema maltophilum*, porins from *Neisseria*, atypical LPS from *Leptospira* species, and *Porphyromonas* species.
- g) TLR-2 selected from the group consisting of Pam₃Cys, HSP70, *Staphylococcus epidermidis*, *Trypanosoma cruzi*, *Leptospira interrogans*, and *Porphyromonas gingivalis*;
- h) TLR-3 selected from the group consisting of viral double-stranded RNA and poly dI:dC;
- i) TLR-4 selected from the group consisting of LPS from gram-negative bacteria and its derivatives, HSPs, Taxol, fusion proteins of RSV, envelope protein of MMTV, fibronectin, fragments of fibronectin, oligosaccharides of hyaluronic acid, polysaccharide fragments of heparan sulfate, and fibrinogen;
- j) TLR-4 selected from the group consisting of monophosphoryl lipid (MPLA), HSP60, and HSP70.
- k) TLR-5 from the group consisting of bacterial flagellin;
- l) TLR-6 from the group consisting of di-acyl lipopeptides from mycoplasma;
- m) TLR-7 selected from the group consisting of imidazoquinoline, loxoribine, and broprimine; and

- n) TLR-9 from the group consisting of unmethylated DNA;
- o) TLR-9 selected from the group consisting of CpG-DNA and CpG-PTO oligonucleotides.

Claim 61 (New): The composition of matter of claim 34, wherein a targeting moiety is attached to the liposome.

Claim 62 (New): A method for producing the composition of matter of claim 34, wherein the method of producing the liposomes of (a) – (e) comprises:

- a) forming a suspension of at least one lipid, one or more therapeutic agent, and optionally a first and/or a second adjuvant in a liquid medium and
- b) homogenizing the suspension.

Claim 63 (New): A liposome produced by the method of claim 62.

Claim 64 (New): A method for treating or preventing a disorder, comprising administering a composition of matter of claim 34 to a subject, wherein the disorder is selected from the group consisting of proliferative disease, infectious disease, vascular disease, rheumatoid disease, inflammatory disease, immune disease, and allergy.

Claim 65 (New): The method of claim 64, wherein the proliferative disease is selected from the group consisting of carcinomas of the gastrointestinal or colorectal tract, liver, pancreas, kidney, bladder, prostate, endometrium, ovary, testes, melanoma, dysplastic oral mucosa, invasive oral cancers, small cell and non-small cell lung carcinomas, hormone-dependent breast cancers, hormone independent breast cancers, transitional and squamous cell cancers, neurological malignancies, osteosarcomas, soft tissue sarcomas, hemangiomas, endocrinological tumors, hematologic neoplasias, carcinomas *in situ*, hyperplastic lesions, adenomas, fibromas, histiocytosis, chronic inflammatory proliferative diseases, vascular proliferative disease, and virus-induced proliferative diseases.

Claim 66 (New): The method of claim 65, wherein the proliferative disease is selected from the group consisting of leukemia, lymphoma, myeloproliferative disease, lymphoproliferative disease, neuroblastoma, glioma, and astrocytoma.

Claim 67 (New): The method of claim 64, wherein an adjuvant, or a cytokine, or both are administered prior, simultaneously, or after administration of the liposome or liposomal composition.

Claim 68 (New): The method of claim 67, wherein the adjuvant is selected from the group consisting of unmethylated DNA, alum, bacterial products from the outer membrane of Gram-negative bacteria, synthetic lipopeptide derivatives, lipoarabinomannan, peptidoglycan, zymosan, HSP, dsRNA, synthetic derivatives of dsRNA, polycationic peptides, taxol, fibronectin, flagellin, imidazoquinoline, cytokines with adjuvant activity, oil in water emulsions, ween 80, Span 85, QS-21, non-ionic block polymers, polyphosphazene, BAY R1005, calcitriol, DHEA, [MDP(Gln)-OMe; murapalmitine, polymers of lactic and/or glycolic acid, polymethyl methacrylate, sorbitan trioleate, squalane, stearyl tyrosine, squalene, theramide, and synthetic oligopeptides.

Claim 69 (New): The method of claim 68, wherein the adjuvant is selected from the group consisting of CpG PTO ODN, CpG PO ODN, MPLA, LPS, muramyl dipeptides, derivatives of muramyl dipeptides, Pam₃Cys, HSP 70, Poly I:poly C, GM-CSF, IL-2, IL-6, IL-7, IL-18 type I, IL-18 type II, interferon-gamma, TNF-alpha, MF59 consisting of squalene, Poloxamer 401, saponins, derivatives of saponins, peptides presented by MHC-class II, and poly-L-arginine.